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MICRO-SYNTHESES WITH TRACER ELEMENTS. XVI
THE SYNTHESIS OF HEXESTROL LABELED WITH TRITIUM

by

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MICRO-SYNTHESES WITH TRACER ELEMENTS. XVI. THE SYNTHESIS OF HEXESTROL LABELED WITH TRITIUM

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INTRODUCTION

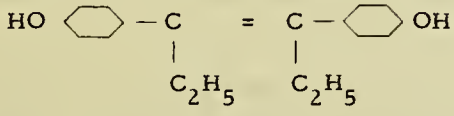
Information was desired regarding the stability of organic compounds containing the isotope tritium in place of hydrogen. Since a higher concentration of radioactivity can be imparted to an organic compound by means of tritium than with almost any other element, this method of labeling should prove to be ideal for those chemical entities which produce profound biologic changes in the living system when present in exceedingly small concentrations.

A group of compounds having such an effect are the sex hormones. Minute amounts of these chemical entities can bring about deep seated physiologic changes in both the male and female.

One of the female sex hormones, "hexestrol" is synthetic in the sense that it does not occur naturally in living organisms, but is a very powerful estrogen. This compound may be easily labeled with tritium and was accordingly chosen for study.

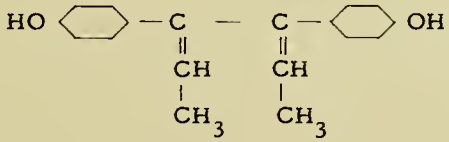
THEORETICAL AND HISTORICAL

Hydrogenation of the unsaturated aliphatic linkage of either "Stilbestrol" (I) (4, 4'-dihydroxy-α, β-diethylstilbene)



(I)

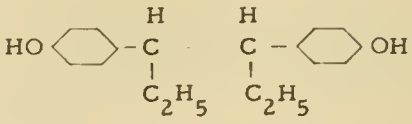
or "Dienestrol" (II) (3, 4-di(p-hydroxyphenyl)-2,4-hexadiene)



(II)

should afford an efficient means of labeling "Hexestrol" (III)

[3, 4-di(p-hydroxyphenyl)hexane]



(III)

with the radioisotope of hydrogen, tritium (H^3). Hexestrol, first isolated from the demethylation products obtained from anethole by Dodds et al[1], displays an extremely high estrogenic activity.

The d-, l-, meso-, and racemic forms of hexestrol are possible since the compound contains two asymmetric carbon atoms. The same workers isolated an isomer of hexestrol melting at 128°C (hexestrol, m.p. 184-5°C) which was a much less potent estrogen. Wessely and Welleba[2] succeeded in resolving isohexestrol (racemic form) into d- and l- forms and accordingly assigned the meso form to hexestrol. An equimolar mixture of the d- and l- forms melted at 129°C, confirming the accuracy of their separation.

Hydrogenation of stilbestrol gave a mixture of isohexestrol and hexestrol; the proportions of the two compounds depended upon the catalyst used and the conditions of the hydrogenation.

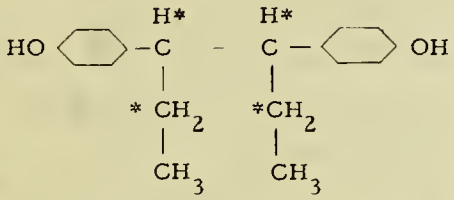
Wessely and Welleba[2] obtained an 88% yield of isohexestrol and a 12% yield of hexestrol when hydrogenation was carried out in acetic acid at 18°C using palladium sponge.

Docker and Spielman[3] claimed a quantitative yield of isohexestrol by the hydrogenation of stilbestrol using either palladium, copper chromite, or Raney nickel catalysts. The high yields of isohexestrol obtained by the hydrogenation of stilbestrol has been confirmed by others[4, 5] and stilbestrol apparently does not seem desirable as a starting material for the labeling with H^3.

Dodds et al reported the successful synthesis of dienestrol and the hydrogenation of the compound to hexestrol in acetone solution using a

palladium catalyst. A quantitative yield was reported. The exact nature of the catalyst, e.g., whether supported or not, was not given.

In view of this work, dienestrol was selected for hydrogenation to hexestrol. The H^3 atoms are located at the positions marked with an asterisk (IV).



(IV)

EXPERIMENTAL

In a series of preliminary experiments, dienestrol (m.p. 231-2°C) was hydrogenated for the purpose of studying the conditions of hydrogenation and procedures for purification of the product. A resume of these experiments is given in Table I.

1. APPARATUS

The apparatus used for carrying out the preliminary experiments is shown in Figure 1. To a high vacuum manifold was attached a mercury manometer, a gasometer and a 25 ml. flat-bottomed reaction flask. The flask contained a short glass enclosed iron rod which served as a magnetic stirrer. Experiments 1, 2, 3, 4, 5, and 9 were carried out in this apparatus.

Since the health hazards from tritium are as yet unknown and since we were advised that tritium was tenaciously adsorbed on glass surfaces, the system shown in Figure 2 was designed and used in the tritium hydrogenation. All contact of tritium with grease was eliminated as much as possible. No semi-ball joints were used throughout the entire system. The system was so arranged that the contaminated portion could be isolated and disposed of after the experiment was completed. A diagram of the apparatus is shown in Figure 2.

The labeling of the dienestrol was carried out according to the following procedure. H_2^3, 0.15 ml., diluted with H_2 was sealed in "U" tube A which contained two "breakoffsky" sections y and z. B was a 25 ml. flat bottomed reaction chamber containing catalyst, solvent, dienestrol and a short glass enclosed iron rod which served as a magnetic stirrer. C, a "U" tube having a 25 ml. capacity was connected to a gasometer D the outlet of which contained a ground glass valve, E. This was used to prevent the accidental flow of mercury out of D and into the system. The system contained constrictions at points marked F, G and H.

In carrying out the hydrogenation, tube A containing H_2^3-H_2 and 2 steel balls and reaction chamber B containing the catalyst, solvent, dienestrol and stirrer were sealed to the system. The contents of B were frozen with liquid N_2 and the entire system was evacuated to 3-5 microns. During this process the mercury rose in the gasometer until stopped by valve E. After being tested for leaks the reaction system was isolated from the exhaust manifold by sealing the constricted points F and G. After the contents in B had melted completely, the "breakoffsky" at Y was broken and the H_2^3-H_2 was allowed to react for 45 min. with the well-stirred reaction mixture. Meanwhile the gas buret D was filled with H_2. The leveling bulb K was raised in order to place a positive pressure on the system, and "breakoffsky" Z was then broken. The steam of H_2 served to carry over into the reaction chamber any residue of H_2^3-H_2 remaining in "U" tube A. The course of the reaction was followed by the decrease in volume of H_2.

After hydrogenation was complete the mercury reservoir K was lowered and tube C and the gas bulb of D were both cooled with liquid N_2. When the solvent from A had disappeared entirely, the system was sealed at H and the reaction flask was removed.

Since the health hazard for tritium is as yet unknown, the glass system was melted apart at points F, G, and H, the tubing connected to the reservoir

closed with clamps and cut, and the portion of the system thus isolated was removed for disposal.

Experiments 6, 7 and 8 were carried out in this type of apparatus.

Platinum oxide was used in Experiments 1, 2 and 3. The catalyst was reduced in the solvent. The water and solvent was then removed in a high vacuum and additional solvent with the dienestrol to be reduced, was introduced into the reaction chamber.

At the completion of the experiment the reaction mixture was filtered thru a medium sintered glass funnel. The solvent was removed under vacuum and the product was also dried under vacuum.

2. CATALYSTS

The Adams platinum oxide catalyst proved not to function well in the reduction of dienestrol. The rate of hydrogenation diminished rapidly and the total hydrogenation was not quantitative. The addition of ferrous sulfate, which sometimes inhibits deactivation of this catalyst, did, in this experiment, both increase the rate and complete the hydrogenation.

Some material insoluble in benzene, the nature of which was not investigated, was formed in Experiments 2 and 3. Since Dodds et al[5] used as catalyst, palladium and since the platinum catalyst gave uncertain results, a catalyst of 10% palladium on carbon was used for the hydrogenation of dienestrol.

Solubility data of pure hexestrol in benzene[6] at 15-16°C indicated that a ratio of 5 ml. of benzene per 100 mg. of hexestrol should give a 90% yield of product upon recrystallization. The yield of material melting between 183-185°C obtained in Experiments 5, 6, 7, 8, and 9 was 72 to 77% of theory. Upon concentration of the mother liquor to 1/5 the original volume an additional 5-7% of product melting at 175-180°C was always obtained.

The second mother liquor obtained in this manner in Experiment 8 was evaporated to a volume of 1.5 ml., refluxed gently to dissolve all material present and cooled. A quantity of substance, 137 mg. (26.7% of theory), melting at 126-8°C, separated in the form of fine, colorless needles. This compound corresponds to isohexestrol (m.p. 128-129°C[2.4]. This compound

was not reported by Dodds and co-workers[5] who hydrogenated dienestrol in acetone solution with palladium catalyst. These investigators either overlooked this fraction, or used a catalyst possessing a different composition from the one used in the present experiments.

Evaporation of the final mother liquor to complete dryness left a colorless residue which melted at 123-125°C (7.1% of theory).

It should be noted that other solvents tried did not give this clean-cut separation of meso-hexestrol from racemic-hexestrol (iso). Dioxane-H_2O and carbon tetrachloride solvents gave an 85% yield of material melting 10°C low for the meso form.

3. SOLVENT OF CRYSTALLIZATION

In all experiments the yield of crude product was in excess of 100%. In no instance did additional drying, even under vacuum decrease the weight.

In those experiments in which absolute ethanol was used as solvent, the excess in weight amounted to approximately 23% of the theoretical (Experiments 1, 3, 9). When acetone was used as solvent the excess weight was about 19.0% of the theoretical. (Experiments 4, 5, 6, 7, 8).

The first assumption was that some inorganic salt was extracted from the catalyst. However, when a weighed quantity of catalyst was extracted either with acetone or with alcohol, the weight of catalyst remained unchanged. No residue remained after evaporation of the solvent. It is significant that the same percentage excess in the weight occurred for each solvent regardless of the amount of material hydrogenated or of the catalyst used.

The possibility that a pinacol was formed from the acetone is not tenable since an increase in weight also appeared with ethanol as solvent. This was further checked by attampting to hydrogenate acetone with the palladium catalyst used. There was no hydrogen uptake over a four hour period.

The excess weight of crude product corresponds closely to one mole of solvent per mole of compound. It then appears likely that a mole of solvent is held as solvent of crystallization.

4. H^3 LABELED HEXESTROL

The reaction chamber containing a mixture of 503 mg. of dienestrol, 54 mg. of 10% palladium on charcoal, and 5 ml. dry acetone was sealed to the

apparatus in the manner described above, Figure 2, and the $H_2{}^3$-H_2 hydrogenation completed. After complete hydrogenation the solvent was removed, as described previously, and the hexestrol was isolated.

The product was redissolved in acetone and the solution was filtered in a medium sintered glass funnel thru a bed of infusorial earth -- "Norite" carbon. This was necessary in order to remove the suspended catalyst. The flask and catalyst were washed with 2 ml. portions of acetone. The total solution, 50 ml., was evaporated to dryness in a vacuum, and completely dried in a vacuum desiccator. The crude product weighed 594.6 mg. (116.2% of theory).

The crude product was purified by recrystallation from 30 ml. of benzene. The crystals were allowed to form at room temperature, and then at 6-8°C (2 hours). The mother liquor was removed and collected in a tared 40 ml. cone with a filter stick. The colorless product after drying in a desiccator under vacuum weighed 386.4 mg. (75.8% of theory) and melted at 184-5°C. This is the meso form commonly called hexestrol and is the isomer of high estrogenic activity.

Evaporating the mother liquor to dryness left a colorless residue weighing 220.8 mg. The residue was dissolved in 5 ml. of benzene with gentle refluxing, then cooled to 6-8°C for 1 hour. The mother liquor was removed with the filter stick as before. The colorless crystalline residue weighed 34.9 mg. (6.8% of theory) and melted at 160-4°C. This undoubtedly is a mixture of hexestrol and isohexestrol.

The second mother liquor was evaporated, under vacuum to a volume of 1-1/2 ml. and then was cooled to 5-6°C for 1 hour. The mother liquor was removed as before and the colorless needles were washed with 0.5 ml. of dry benzene. This fraction after drying weighed 142.8 mg. (28% of theory) and melted at 126-7°C. This fraction is the racemic form called isohexestrol. The mother liquor when evaporated to dryness left a residue weighing 30.2 mg. and which melted at 124-5°C.

DISCUSSION OF RESULTS

It was surprising to observe that the rate of hydrogenation was much more rapid when the H_2^3-H_2 mixture was used than in the last trial run using H_2, Experiment 8, in which the conditions were identical in every respect.

The two rates of hydrogenation are shown in Figure 3 in which quantities of hydrogen consumed per unit times are compared.

Solvent, catalyst and hydrogen from the same stocks in the same apparatus was used throughout both experiments. The hexestrol obtained in this experiment possessed 1 mc. of radioactivity per mg.

SUMMARY

The estrogen, hexestrol, labeled with the radioactive isotope of hydrogen, tritium, has been prepared.

Both meso- and racemic forms of hexestrol were obtained by the hydrogenation of dienestrol with a palladium on carbon catalyst.

The presence of a small amount of tritium exerted a very significant influence upon the rate of consumption of ordinary hydrogen under conditions which were otherwise identical.

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3. Docker and Spielman, J. Am. Chem. Soc., 62, 2163 (1940).
4. Campbell, Dodds and Lawson, Proc. Roy. Soc. (London) B128, 253 (1939-40).
5. Dodds, Goldberg, Lawson, Robinson, Proc. Royal Soc. (London) B-127, 140 (1939).
6. Cheymol and Carayon - Gentil. Bull. Soc. Chim. Biol. 28, 136 (1946) C. A. 41, 3518e.

TABLE I

Exp.	Catalyst	Solvent	Dienestrol Wt. mg.	Hexestrol Theory Wt.	Crude Product Mg.	% Theory	M.P.	Recryst'd. From.	Fraction 1 Mg.	M.P	Fraction 2 Mg.	M.P.	Fraction 3 Mg.	M.P.	Residue
#1	0.059. PtO_2	Abs. EtOH	50.1	50.8	62.8	123.5	-	Dioxane $-H_2O$	38.7						
#2	0.059. PtO_2	Abs. EtOH	43.4	43.8	46.3	105.5	-	Benzene	All product did not dissolve in Benz.						
#3	0.059. PtO_2 0.0169. $FeSO_4$	Abs. EtOH	52.7	53.5	66.1	123.5	-	This product was used to explore solvents for recrystallization.							
#4	0.119. 10% Pd on C	Acetone	52.2	53	59.9	113.1	169-170	CCl^4 Benzene	45.7	174 184-5					
#5	0.119. 10% Pd on C	Acetone	110.3	111.9	133.1	119.0	162-5	Benzene	80.9	185-6	7.7	175-7°			
#6	0.059. 10% Pd on C	Acetone	102.7	104.1	123.2	118.3	-	Benzene	80.3	182-4	6.7	176-8°			24.6
#7	0.059. 10% Pd on C	Acetone	204.9	210.0	251.2	119.5	-	Benzene	156.2	183-5					
#8	0.059. 10% Pd on C	Acetone	500.6	513.7	613.9	119.5	160-1	Benzene	395.6	184-5	28.7	176-180°	137.0	126-8°	36.7 M.P.123-5
#9	0.059. 10% Pd on C	Abs. EtOH	98.7	100	123.8	123.8	161-6	Benzene	76.0	184-5	6.8	142-55°			30.9

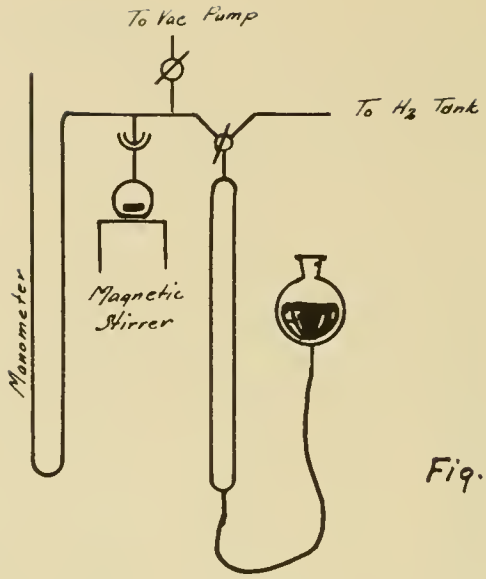

Fig. 1

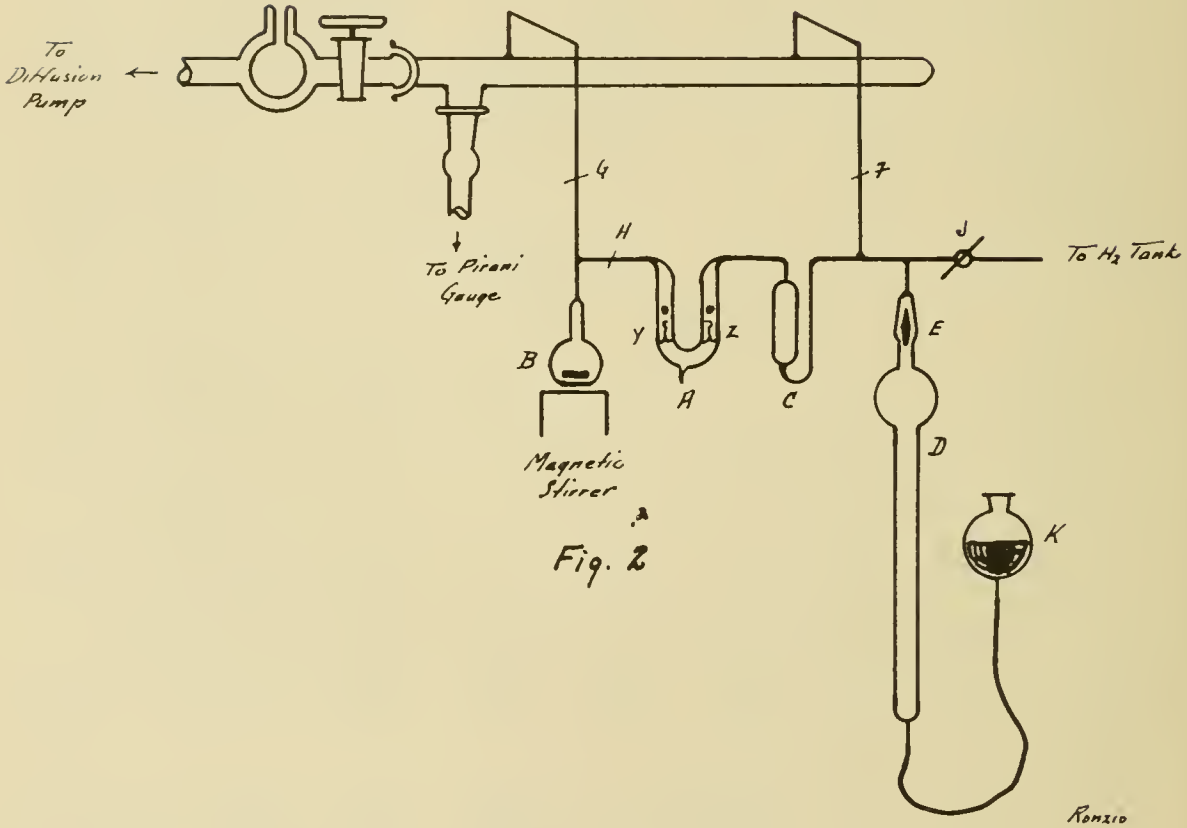

Fig. 2

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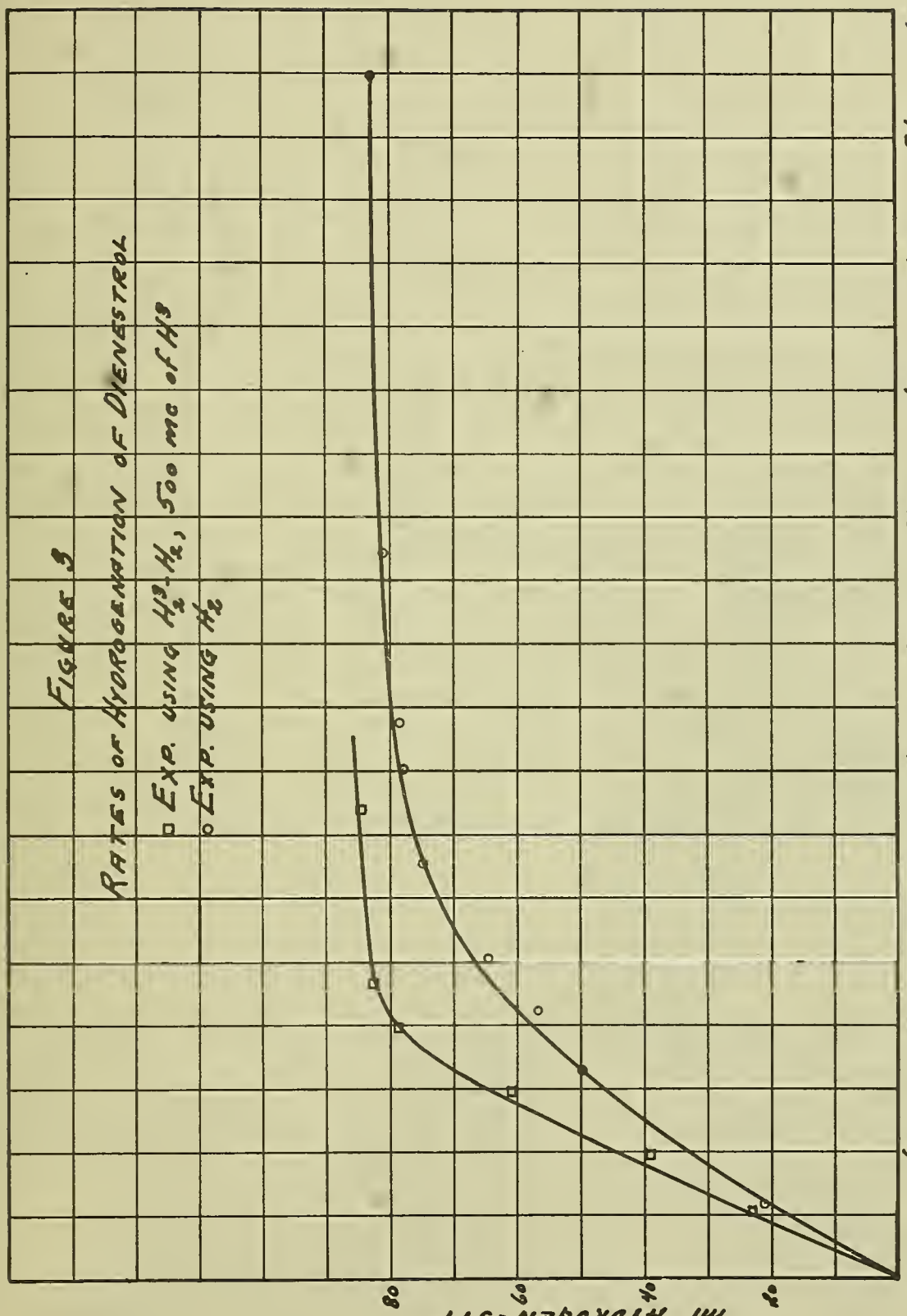
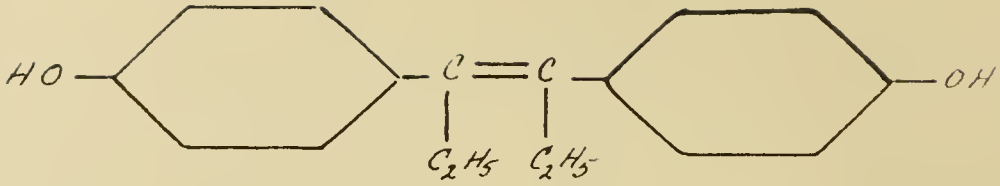


FIGURE 3
RATES OF HYDROGENATION OF DIENESTROL
□ EXP. USING H_2^{18}-H_2, 500 mg OF H_2^{18}
○ EXP. USING H_2
ml HYDROGEN - STP
TIME IN MINUTES
40
60
80
120
180
240
300
360
420
480
540
600



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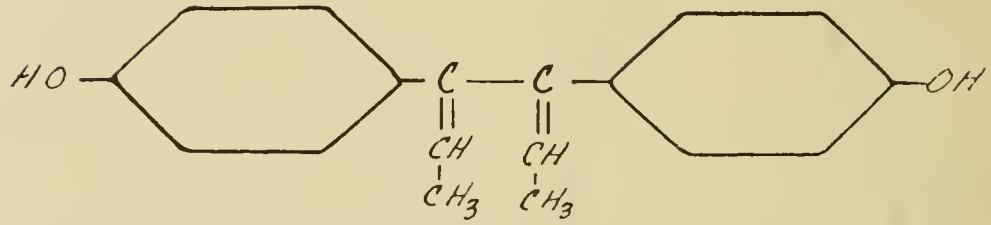

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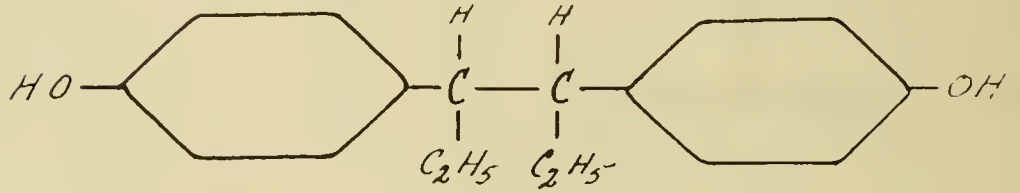

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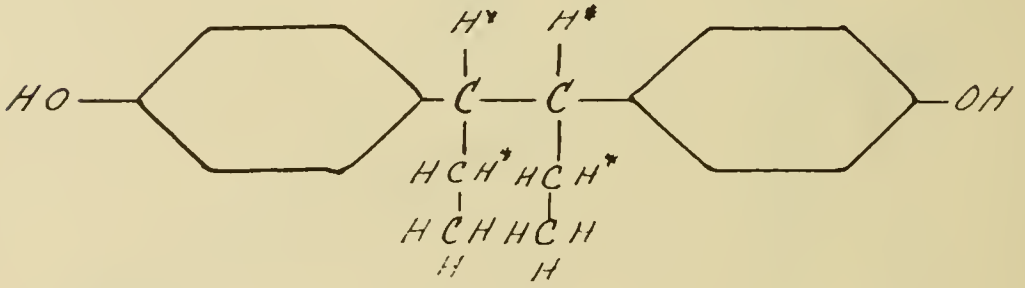

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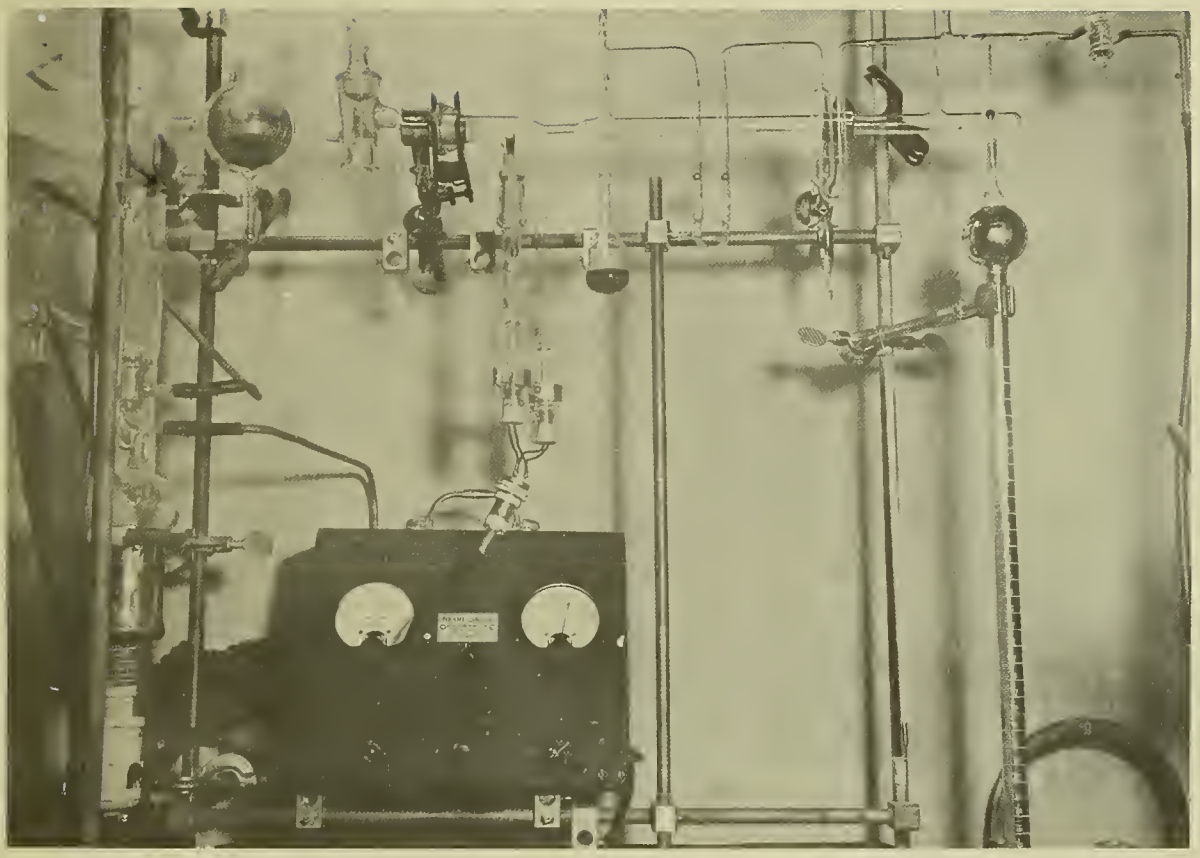

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